

## Osteocalcin levels in gingival crevicular fluid periodontitis patients with and without type 2 Diabetes Mellitus

Muhammad Nabeel Wildan\*, Ahmad Syaify\*\*, Dahlia Herawati\*\*

\* Magister student of Dental Clinic, Periodontics Study Program, Universitas Gadjah Mada

\*\* Department of Periodontics, Faculty of Dentistry, Universitas Gadjah Mada

Correspondence: [ahmad.syaify@ugm.ac.id](mailto:ahmad.syaify@ugm.ac.id)

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Osteocalcin; gingival crevicular fluid; periodontitis; Diabetes Mellitus

### ABSTRACT

**Background:** Diabetes Mellitus (DM) is a systemic disease with a high prevalence in Indonesia. Periodontitis is a complication of DM with frequent occurrences. Periodontitis with DM causes greater bone damage than non-DM periodontitis because its conditions can affect osteoblast and osteoclast activity. Osteocalcin (OC), mostly synthesized by osteoblasts, regulates the activity of bone formation and turnover so that it can be a marker of bone formation and turnover to see the severity of the disease. OC can be found in gingival crevicular fluid (GCF). This study aimed to determine osteocalcin levels in gingival crevicular fluid periodontitis patients with uncontrolled type 2 Diabetes Mellitus and non-Diabetes Mellitus.

**Methods:** Samples used in this study were gingival crevicular fluid taken from 20 samples of periodontitis patients with DM type 2 (T2DMP) and 20 samples of periodontitis non-DM patients. The OC levels were analyzed using ELISA Kit Osteocalcin then statistical tests were carried out.

**Result:** The results showed that the OC level of the T2DMP group was lower with mean of  $0.369 \pm 0.140$  while the periodontitis non-DM group was  $0.664 \pm 0.141$ .

**Conclusion:** The OC levels of periodontitis patients with DM are lower than periodontitis patients with non-DM.

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## INTRODUCTION

A metabolic disorder known as diabetes mellitus (DM) is characterized by persistent infections and impaired wound healing. While patients with controlled diabetes are in better physical condition, those with uncontrolled diabetes frequently experience impaired wound healing and a higher likelihood of infection.<sup>1</sup> Diagnostic criteria for DM are obtained by examining HbA1c with a more than 6.5% value.<sup>2,3</sup> According to the World Health Organization, diabetes is classified into six categories, one of which is type 2 DM. DM is highly prevalent, with more than 382 million people worldwide experiencing DM disease.<sup>4</sup> Indonesia is one of the countries with a relatively high prevalence of DM, and it is predicted to increase to 21.3 million people in 2030.<sup>5</sup> DM with periodontitis complications had a high prevalence rate of up to 75%.<sup>6</sup>

Patient who had periodontitis and DM in the same time had a tendency to destruction of periodontal tissue and alveolar bone higher than in patients without diabetes.<sup>7</sup> DM can affect the activity of osteoblasts, osteoclasts, and bone turnover, affecting the severity of periodontitis.<sup>8</sup> This condition can result in more severe damage to the periodontal tissue if left untreated, also can affect aesthetics and function to a tooth.<sup>9</sup> Periodontitis can occur in all age groups (adults, children, and adolescents) but it is most often found in adults. Approximately 80% of all cases of periodontitis are characterized by periodontal pocket formation and gingival recession. It is described as a disease resulting in inflammation in the supporting tissues of the teeth, progressive attachment loss, and destruction of the alveolar bone.<sup>10</sup> Osteoclasts and osteoblasts have an important role in bone resorption and formation.<sup>11</sup> During the remodeling process, osteoclasts have continuous bone resorption, followed by new bone formation by

osteoblasts. Information about the status of bone remodelling can be an early indicator of bone damage leading to periodontal disease.<sup>12</sup> Osteocalcin, which is mostly synthesized by osteoblasts, odontoblasts, and hypertrophic chondrocytes, regulates the activity of bone formation and bone turnover. Osteocalcin can be an effective bone turnover marker when bone resorption and formation occur simultaneously.<sup>8</sup> Many studies have been conducted on oral fluids that detect various biomarkers in oral fluids, such as saliva and gingival crevicular fluid (GCF) of periodontitis patients. Examination of biomarkers in oral fluids helps in the diagnosis, therapy, and prognosis of a disease.<sup>13</sup> The GCF contains various bone-related biomarkers that can describe a state of periodontal disease, including osteocalcin.<sup>14</sup> The level of osteocalcin in the GCF and saliva of periodontitis patients is related to the disease progression and severity.<sup>15</sup> Research conducted by Joseph et al.<sup>16</sup> demonstrated a correlation between osteocalcin in saliva and periodontal health parameters in periodontitis patients. Serum osteocalcin in periodontitis patients was lower than that of healthy patients.<sup>17</sup> The serum level of osteocalcin in DM patients is lower than in healthy patients. This shows that osteocalcin can be used as a good biomarker in predicting the development of periodontitis disease related to DM.<sup>18</sup> Moussa<sup>19</sup> said that osteocalcin in GCF has a higher accuracy level than osteocalcin in serum and saliva of periodontitis patients, but further research is needed on periodontitis patients with DM. This is necessary in determining the prognosis and the right type of treatment for periodontitis patients with DM by knowing their osteocalcin level considering the number of patients who experience it and severity that can occur.

## RESEARCH METHODS

### Population Study

The subject study was screened at the Dental hospital UGM Prof. Soedomo and the KORPAGAMA Family Physician Clinic. A total of 40 subjects, regardless of gender, were involved in this study. Subjects were defined as periodontitis with uncontrolled type-2 DM (T2DMP) and periodontitis non-DM based on clinical examination and HbA1C test. This study was performed after approval from the Faculty of Dentistry Universitas Gadjah Mada Ethics Committee (decision no: 195/KE/FKG-UGM/EC/2022). An informed consent form was given to each subject. Periodontitis non-DM (n=20) included subjects with probing depth (PD)  $\geq$  4mm and clinical attachment loss (CAL)  $>$ 3mm (Ko et al. 2021), whereas periodontitis with uncontrolled type 2 DM group (T2DMP) (n=20) included subjects with periodontitis criteria and the result of HbA1C test more than 7%.

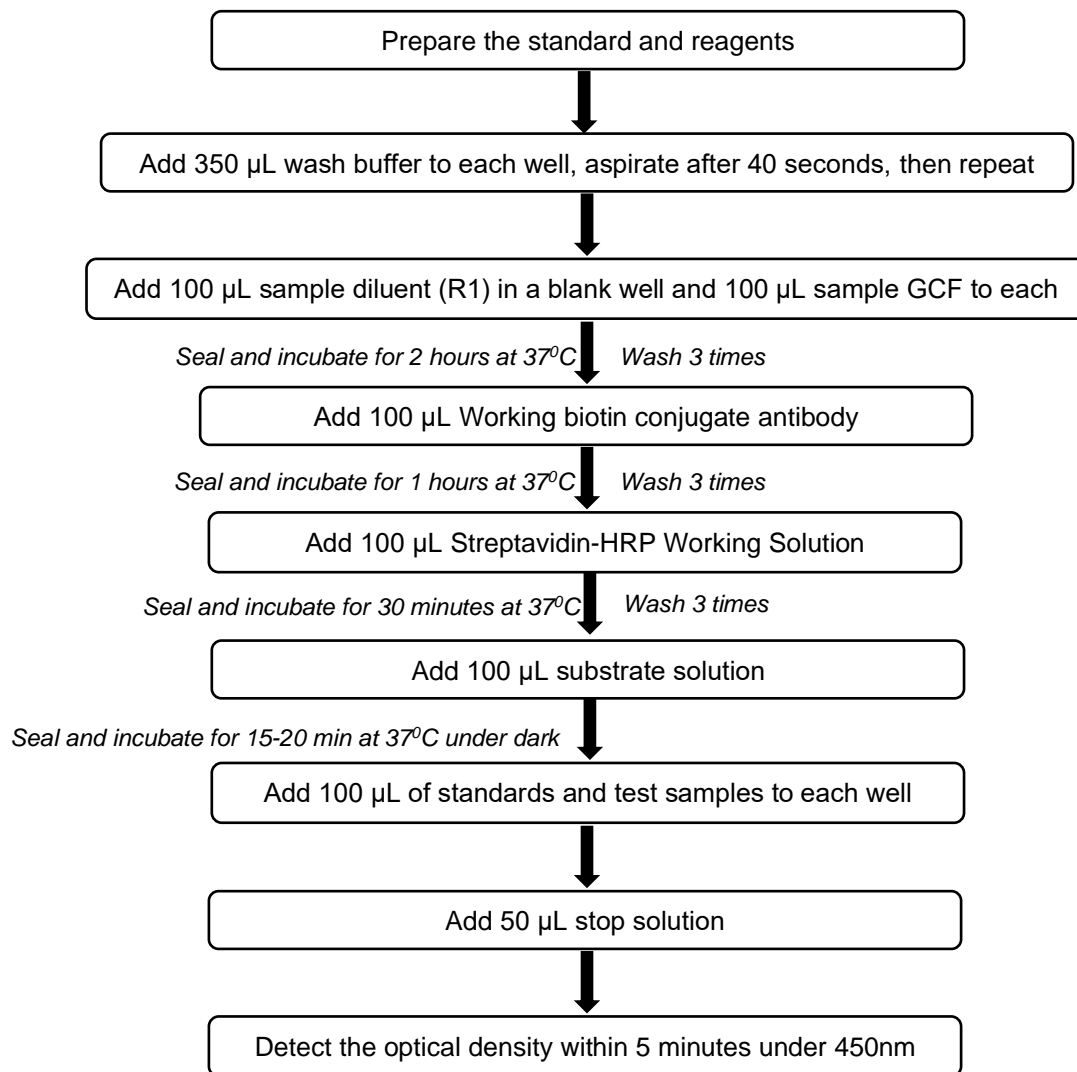
### Sampling of gingival crevicular fluid (GCF)

The supragingival plaque was carefully cleaned using a cotton pellet and dried up with a three-way syringe to prevent contamination from saliva. Sulcular fluid was collected from 3 sites in one tooth per patient (mesial, facial, and distal) using paper points inserted into the sulcus between the teeth and the gingival margin to a depth of 2

mm. These paper points were left for 5 minutes in the gingival sulcus. Subsequently, the sulcular fluid was placed in an eppendorf tube, immersed in 100 $\mu$ L of PBS solution, and centrifuged at 1000 rpm for 20 minutes at 40C. Finally, all the samples were stored in the refrigerator at -800C.

### Osteocalcin (OC) assay

OC measurements according to ELISA manufacturer's instructions. All reagents and samples were placed at room temperature before being used. The procedure follows: Add 350  $\mu$ L wash buffer to each well, aspirate each well after holding for 40 seconds, and repeat the process twice. Add 100  $\mu$ L sample diluent (R1) in a blank well and 100  $\mu$ L sample GCF to each well, then covered with adhesive sealer and incubated for 2 hours at 37 $^{\circ}$ C, then repeat aspiration/wash three times. Next, add 100  $\mu$ L working biotin conjugate antibody and incubate for 1 hour at 37 $^{\circ}$ C, repeat aspiration/wash three times. After washing, add 100  $\mu$ L working streptavidin-HRP and incubate for 30 minutes at 37 $^{\circ}$ C, then repeat aspiration/wash three times. Furthermore, 100  $\mu$ L substrate solution was added and incubated for 15-20 minutes at 37 $^{\circ}$ C in the dark room. Finally, add 50  $\mu$ L stop solution and detected optical density for 5 minutes with a 450 nm microplate reader.



### Statistical Analysis

IBM SPSS Statistics 25.0 software is used to process data. The normality test was performed using Shapiro-Wilk. Furthermore, the homogeneity test was carried out with Levene's test. Then a statistical test was carried out using the parametric T-test with a significance level of 95%.

### RESULTS

A total of 40 patients participated in this study, with an age range of 50-70 years for the T2DMP group and 30-50 years for the non-DM

periodontitis group (Table 1). Examination of pocket depth and clinical attachment loss was performed in all patients, and it was found that the T2DMP group had higher PD and CAL results than the periodontitis non-DM group. In the T2DMP group, the most pocket depth was 5-10 mm in 14 patients, and the most CAL was 5-10 mm in 10 patients, while in the periodontitis non-DM group, the most pocket depth occurred at <5mm in 18 patients, and the most CAL was 5- 10 mm in 14 patients (Table 2).

Table 1. Characteristics of the research samples

Group	N	Age (years)	Sex	
			Male %	Female%
T2DMP	20	50-70	30	70
Periodontitis non- DM	20	30-50	45	65

Table 2. Periodontal Tissue Examination

	T2DMP	Periodontitis non-DM
<b>Pocket Depth</b>		
<5 mm	5	18
5-10 mm	14	2
>10 mm	1	-
<b>Attachment Loss</b>		
<5 mm	1	14
5-10 mm	10	6
>10 mm	9	-

The Mean  $\pm$  SD of OC was significantly lower in T2DMP group as shown in Table 3 compared to the periodontitis non-DM group at P-value <0.005.

Table 3. Periodontal Tissue Examination

Group	N	Mean	Deviation Std.	P-value
T2DMP	20	0.369	0.140	<0.001*
Periodontitis Non-DM	20	0.664	0.141	<0.001*

\*There is a significant difference

## DISCUSSION

DM is an important risk factor in the development and severity of periodontitis which results in damage to the supporting tissues and bones that form attachments around the teeth.<sup>20</sup> Hyperglycaemic conditions in DM patients can increase bacterial growth and interfere with the immune response, causing greater periodontal tissue damage.<sup>21,26</sup> Periodontal examination shows that pocket depth and CAL was higher in the T2DMP group compared to the periodontitis non-DM group, means that periodontitis patients with

DM had more severity in their periodontal tissue damage.

This study used gingival crevicular fluid samples in patients with uncontrolled T2DMP and periodontitis non-DM patients. Gingival crevicular fluid (GCF) is an inflammatory exudate that seeps into gingival sulcus. It will be increase in pathological condition such as bone or connective tissue damage.<sup>22</sup> Sample of GCF can potentially be used to not only as biomarker in determining diagnosis, prognosis, and treatment plans in

treating periodontal disease, but also determine responses to a therapeutic intervention.<sup>23</sup> This study assessed osteocalcin levels in GCF samples due to their ability as biomarkers in periodontal disease. This is following the research of Yıldırım et al.<sup>24</sup> which said that osteocalcin can be used as a bone formation marker. Gingival crevicular fluid can show more accurate osteocalcin values compared to serum or saliva.<sup>19</sup>

The research subjects used the age range in the T2DMP group namely 50-70 years and 30-50 years in the periodontitis non-DM group. Chronic periodontitis is commonly found in the age range of 30 – 90 years.<sup>25</sup> Increasing age has a close relationship with an increase in blood sugar.<sup>26</sup> Wallis et al.<sup>27</sup> said that severity of periodontitis increases with age. Someone with age of more than 30, the body would experience physiological changes. As getting older, the less body functions will decrease so that it will be more susceptible to various diseases.<sup>28</sup>

The results showed that the osteocalcin level in the T2DMP group was 0.369, while the periodontitis non-DM group was 0.664. Previous studies have reported lower levels of osteocalcin in DM samples than in healthy samples. The results of this study are in accordance with previous research conducted by Liu et al.<sup>29</sup> The researchers compared serum osteocalcin in DM samples and healthy samples with lower results in serum osteocalcin in DM samples. The same thing was found in the study by Naguib et al.<sup>30</sup> where serum OC in DM patients is lower than in healthy patients.

Hyperglycaemia in type 2 Diabetes Mellitus causes pro-inflammatory mediators such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-18 and increases the RANKL/OPG ratio, resulting in large bone resorption.<sup>31,32</sup> High pro-inflammatory mediators increase lipid peroxidation and dyslipidaemia resulting in osteoclastogenesis. High TNF- $\alpha$  levels

increase the RANK/osteoprotegerin (OPG) ratio which cause increasing bone resorption. Chronic inflammation stimulates the expression of proapoptotic genes such as bcl-2-like protein (Bax). Decreased expression of genes that stimulate osteoblast formation, such as Fos-related antigen and Runt-related transcription (RUNX2) which results in increased bone damage.<sup>33</sup>

DM patients with more than 10 years of suffering have a higher disease severity.<sup>26</sup> The longer person suffers from DM, the longer accumulation of AGE increases, which can result in greater damage to the periodontal tissue.<sup>34</sup> Many studies have shown chronic hyperglycaemia and metabolic intermediates such as inflammatory factors, reactive oxygen species (ROS), and Advance Glycation End Products (AGEs) as factors that result in periodontal tissue damage.<sup>29</sup> High glucose levels can cause damage to the periodontal tissue indirectly through AGE's. The glycation product will bind to its receptor (RAGE). The increased binding between RAGE and AGE's activates nuclear transcription factor- $\kappa$ B (NF- $\kappa$ B). This results in increased expression of receptor activating NF- $\kappa$ B ligand (RANKL), which mediates osteoclastogenesis.<sup>33</sup>

AGE's accumulation can also stimulate interleukin (IL), such as IL-6, decreasing osteoblast proliferation and activity while increasing osteoclast activity.<sup>33</sup> Other studies have explained that IL-6 reduces the formation and function of osteoblasts and reduces the number of osteoblasts in DM patients. Lower osteocalcin levels indicate this in type 2 DM patients compared to non-DM patients.<sup>32</sup>

In DM, RAGE, and AGE's bonds also increase the production of reactive oxygen species (ROS).<sup>6</sup> High ROS conditions decrease function and activity of osteoblast. Ma et al.<sup>35</sup> said the accumulation of ROS in type 2 DM resulted in mitochondrial dysfunction and apoptosis in

osteoblasts. This is supported by other studies that said excessive ROS production due to high blood sugar damages osteoblasts.<sup>29</sup> In DM patients, osteocalcin has decreased secretion due to impaired function and activity in osteoblasts.

## CONCLUSION

Based on the results of the study, it can be concluded that the levels of osteocalcin in the gingival crevicular fluid of periodontitis patients with uncontrolled T2DM are lower than in periodontitis non-DM patients. Conversely, the destruction of periodontal tissue in patients with uncontrolled T2DM was higher than periodontitis non-DM patients.

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## REFERENCES

1. Newman MG, Takei HH, Klokkevold PR, Carranza M, *Newman and Carranza's Clinical Periodontology*. 13<sup>th</sup> Ed. China: Elsevier; 2018.
2. Basit A, Fawwad A, Abdul BKA, Waris N, Tahir B, Siddiqui IA, Glycated hemoglobin (HbA1c) as diagnostic criteria for diabetes: the optimal cut-off points values for the Pakistani population; a study from second National Diabetes Survey of Pakistan (NDSP) 2016–2017 *BMJ Open Diabetes Research and Care*, 2020; 8: 1-8.
3. Wulandari IAT, Herawati S, Wandu IN, Gambaran Kadar HbA1c Pada Pasien Diabetes mellitus Tipe II di RSUP Sanglah Periode Juli-Desember 2017, *Jurnal Medika Udayana*, 2017; 9(1): 71-75.
4. World Health Organization (WHO), *Classification of Diabetes Mellitus*; 2019.
5. Savitri E, Sudarman S, Nur NH, Eating Pattern Relationship with Events Diabetes Mellitus Type 2 In the Working Area of The Pertiwi Health Center, Makassar City, *Pancasakti Journal of Public Health Science and Research*, 2021; 1(1): 30-38.
6. Widyawati H, Sudibyo S, Failasufa H, Compliance Relation of Antidiabetic Drug Consumption with Periodontal Network Health in Type II Diabetes Mellitus Prolanis Patients: Case Study at Puskesmas Mranggen III. *Magna Medika*, 2018; 2(4): 1-8.
7. Rizkiyah M, Oktiani BW, and Wardani IK, Prevalensi dan Analisis Faktor Kejadian Gingivitis dan Periodontitis Pada Pasien Diabetes Melitus (*Literature Review*), *Dentin Jurnal Kedokteran Gigi*, 2021; 5(1): 32-36.
8. Daily ZA, Mohammed AN, Periodontal Health Status and Assessment of Osteocalcin levels in Saliva of Diabetic Patients and Systematically Healthy Person (Comparative study). *Journal of Baghdad College of Dentistry*, 2017; 29(1): 98-96.
9. Danastri AA, Suryono, Murdiastuti K, The Influence Between Injectable Platelet-Rich Fibrin and Platelet-Rich Plasma Towards Gingival Fibroblast Cell Proliferation, *ODONTO Dental Journal*, 2021; 8(2): 25-31.
10. Dibart S, Dietrich T, *Practical Periodontal Diagnosis and Treatment Planning*, Wiley-Blackwell; 2010.
11. Hidayat R, Muhtar M, Pangestu MD, Effect of Hydrogel Provision of SHED and PRP Combination on Osteoclast Decreasing in The Alveolar Bone of Male Wistar Rat (*In Vivo*), *ODONTO Dental Journal*, 2021; 8(2): 113-118.
12. Ram VS, Parthiban P, Sudhakar U, Mithradas N, Prabhakar R, Bone biomarkers in Periodontal Disease: A Review Article. *Journal of Clinical and Diagnostic Research*, 2015; 9(1): ZE07-ZE10.
13. Aydin T, Dilsiz A, Measurement of Oncostatin M, Leukemia Inhibitory Factor, and Interleukin-11 Levels in Serum, Saliva, and Gingival Crevicular Fluid of Patients with Periodontal Diseases, *Meandros Medical and Dental Journal*, 2021; 22: 242-251.
14. Bibi T, Khurshid Z, Rehman A, Imran E, Srivastava C, Shrivastava D, Gingival Crevicular Fluid (GCF): A Diagnostic Tool for the Detection of Periodontal Health and Diseases, *Molecules*, 2021; 26(1208): 1-16.
15. Shazam H, Shaikh F, Hussain Z, Majeed MM, Khan S, Khurshid Z, Evaluation of Osteocalcin Levels in Saliva of Periodontitis Patients and Their Correlation with the Disease Severity: A Cross-Sectional Study, *European Journal of Dentistry*, 2020; 14(3): 352-359.
16. Joseph B, Mukhtar AJ, Mohasin AK, AlQahtani SM, Mohammed A, Salivary Osteocalcin as Potential Diagnostic Marker of Periodontal Bone Destruction among Smokers, *Biomolecules*, 2020; 10(3): 2-16.

17. Dogan B, Dede O, The Effect of Bisphosphonates and Low-Dose Doxycycline Therapy in Diabetics with Periodontitis: A Review, *Journal of Dental Faculty of Atatürk University*, 2016; 16 (2572): 65-79.
18. Salman ZA, Ghudaib KK, Fadhil R, Evaluating Osteocalcin in serum male patients with type 2 Diabetes mellitus and periodontitis, *Eurasian Chemical Communication*, 2021; 4:295-302.
19. Moussa SG, Evaluation of locally delivered 1.2% Atorvastatin gel versus 2% melatonin gel as adjunctive to non-surgical periodontal therapy on GCF osteocalcin level in stage II periodontitis patients: a randomized controlled trial, *Future Dental Journal*, 2021; 7(1): 43-48.
20. Pratiwi, AI, Periodontitis as early detection of diabetes mellitus: a literature review. *Intisari Sains Medis*, 2020; 11(1): 108-111.
21. Ahmadinia AR, Rahebi D, Mohammadi M, Mousa G, Jafari A, Esmailzadeh F, Rajabi A, Association between type 2 diabetes (T2D) and tooth loss: a systematic review and meta-analysis. *BMC Endocrine Disorders.*, 2022; 22(100): 1-20.
22. Kurniawati I, Pujiastuti P, Dharmayanti AWS, Kadar Kalsium (Ca) dalam Cairan Krevikular Gingiva Pada Penderita Periodontitis Kronis, *ODONTO Dental Journal*, 2015; 2(2): 9-13.
23. Alassy H, Parachuru P, Wolff L, Peri-Implantitis Diagnosis and Prognosis Using Biomarkers in Peri-Implant Crevicular Fluid: A Narrative Review, *Diagnostics*, 2019; 9(214): 1-14.
24. Yıldırım HS, Ateş M, Gün IO, Kuru B, Çakırer B, Kuru L, Osteocalcin and Cross-Linked C-Terminal Telopeptide of Type 1 Collagen in Gingival Crevicular Fluid during Piezocision Accelerated Orthodontic Tooth Movement: A Randomized Split-Mouth Study, *Nigerian Journal of Clinical Practice*, 2023; 26(4): p470-477.
25. Eke PI, Borgnakke WS, Genco RJ, Recent epidemiologic trends in periodontitis in the USA, *Periodontology 2000*, 2020; 82(1): 257-267.
26. Cahyono TD, Purwanti OS, Hubungan Lama Menderita Diabetes Melitus Dengan Nilai Ankle Brachial Index, *Jurnal Berita Ilmu Keperawatan*, 2019; 12(2): 65-71.
27. Wallis C, Holcombe LJ, A Review of the Frequency and Impact of Periodontal Disease in Dogs, *Journal of Small Animal Practice*, 2020, 61(9): p529-540.
28. Mildawati, Diani N, Wahid A, Hubungan Usia, Jenis Kelamin dan Lama Menderita Diabetes dengan Kejadian Neuropati Perifer Diabetik, *Caring Nursing Journal*, 2019; 3(2): 31-37.
29. Liu J, Cheng Q, Wu X, Zhu H, Deng X, Wang M, Yang S, Xu J, Chen Q, Li M, Icaritin Treatment Rescues Diabetes Induced Bone Loss via Scavenging ROS and Activating Primary Cilia/Gli2/ Osteocalcin Signaling Pathway. *Cells*, 2022; 11(4091): 1-20.
30. Naguib M, Ali N, Elsaraf N, Rashed L, Azzam H, Does Serum Osteocalcin Level Affect Carotid Atherosclerosis in Post-Menopausal Diabetic Females? A Case-Control Study, *International Journal of General Medicine*, 2022; 15:4513–4523.
31. Bastos AS, Graves DT, Loureiro AP, Lipid Peroxidation Is Associated with the Severity of Periodontal Disease and Local Inflammatory Markers in Patients with Type 2 Diabetes. *The Journal of clinical endocrinology and metabolism*, 2012; 97(8): E1353-E1362.
32. Jiao H, Xiao E, Graves DT, Diabetes and its Effect on Bone and Fracture Healing, *Current Osteoporosis Reports*, 2015; 13(5): 327-335.
33. Rathinavelu S, Guidry-Elizondo C, Banu J, Review Article Molecular Modulation of Osteoblast and Osteoclast in Type 2 Diabetes, *Journal of Diabetes Research*, 2018; Vol. 2018: 1-11.
34. Rahmi AS, Syafrita Y, Susanti R, Hubungan Lama Menderita DM Tipe 2 dengan Kejadian Neuropati Diabetik, *Jambi Medical Journal*, 2022; 10(1): 20-25.
35. Ma H, Wang X, Zhang W, Li H, Zhao W, Sun J, Yang M, Melatonin Suppresses Ferroptosis Induced by High Glucose via Activation of the Nrf2/HO-1 Signaling Pathway in Type 2 Diabetic Osteoporosis, *Oxidative Medicine and Cellular Longevity*, 2020; Vol. 2020: 1-18.